SEX INSTABILITY IN RICINUS

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CHANGES in sex expression occur occasionally in individuals of different species of plants. These changes have been attributed, and are often due, to fleeting non-hereditary variations. However, studies of sex expression, during the past five years, have revealed that similar changes may be associated with hereditary instability. In this paper, a description is given of the phenotypic and breeding behavior of various sex forms in *Ricinus communis* L., the castor-oil plant, with particular reference to changes which occur during development.

GROWTH AND FLOWERING HABIT

The habit of growth and flowering of Ricinus is presented diagrammatically in figure 1A. The plant is determinate; the main stem first and later all subsequent shoots terminate in a floral cluster. New shoots can be produced from practically all nodes, but, with few exceptions, branching begins only after the primary inflorescence has appeared at the apex of the main stem. The number and length of internodes preceding the primary inflorescence determine whether a given plant is early or late, dwarf or giant. The castor-oil plant is potentially a perennial tree. A single individual of an early variety may bear over 200 inflorescences during its first year of growth and, under favorable conditions, it will continue to bloom for several years.

This monotypic genus ($2n = 20$) is primarily wind-pollinated and all known variants, including previously described species, intercross readily and produce fertile offspring. The plant is typically monoecious and the inflorescence is racemose. The raceme (fig. 2, A) is made up of a main axis and alternate compound branches which usually bear unisexual flowers; pistillate flowers are borne on the upper branches of the raceme and staminate flowers on the lower branches. The ratio between pistillate and staminate flowers in the racemes of a given plant is the index of sex tendency of that particular plant. In the common monoecious varieties the ratio between pistillate and staminate flowers is about 1:2. Races having higher or lower ratios manifest stronger female or stronger male tendencies respectively. The number of flowers per raceme varies considerably among different strains but it is usually over 120.

SEX VARIATION

Besides extreme racial differences in sex tendency, there are female variants among all castor populations. Instead of bearing both pistillate and staminate flowers, as do racemes of ordinary monoecious plants (fig. 1, A), at least some of the racemes of these female individuals bear pistillate flowers only (fig. 1, C–F). ROXBURGH (1874) discovered a female castor tree in India which led him to believe that there exist dioecious species in this genus. JOSHI (1926) and, later, other workers reported on the occurrence of wide variations in sex tendency among castor inflorescences, including strictly pistillate racemes. What is the nature of these variations?

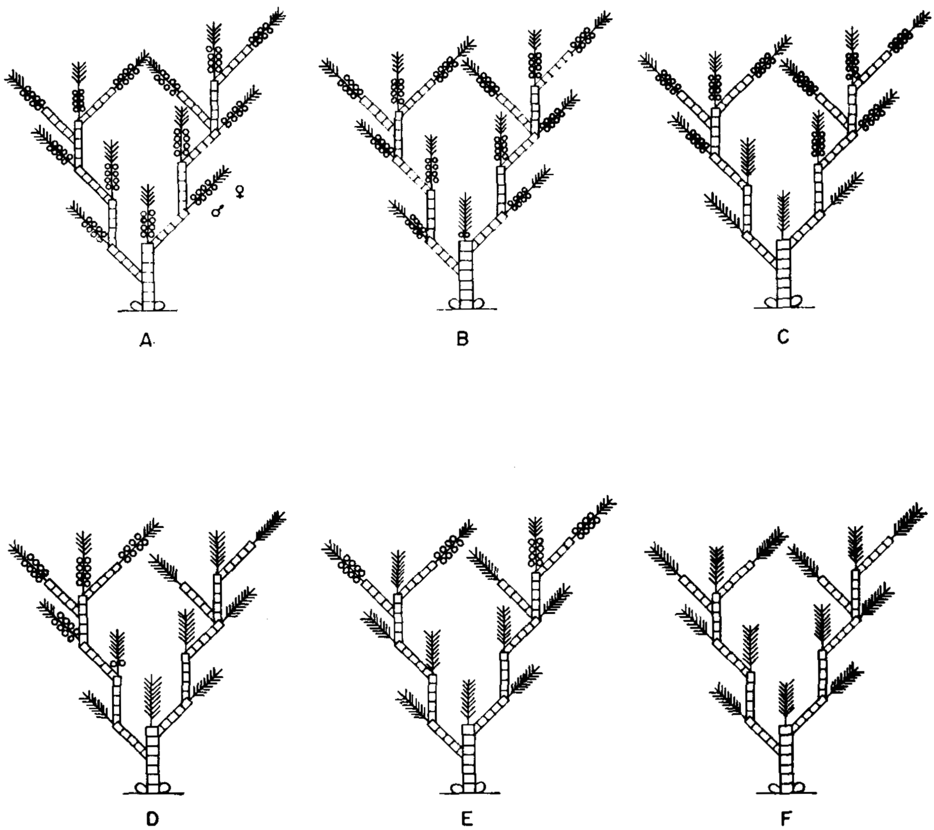

FIGURE 1.—Diagrams of some developmental patterns of sex expression in Ricinus. A, a monoecious individual; B to E, sex reversals; F, strictly female at this stage, but capable of reverting to monoecism depending on genotype.

Sex tendency in castors is subject to extreme non-genetic fluctuations. Female tendency is relatively strong in young plants, especially in primary racemes, and under conditions of moderate temperatures, moderate vegetative activity, and high level of nutrition. Furthermore, female tendency may increase most conspicuously following severe pruning of well established plants. In contrast, male tendency is relatively strong in old declining trees and under conditions of very high or fairly low temperatures, high vegetative activity, and low level of nutrition. While a combination of extremely high temperature and low level of nutrition may lead to a temporary state of complete maleness in plants of some varieties, certain cultural conditions, notably pruning, may bring about a temporary state of complete femaleness in races which ordinarily give a high pistillate-staminate ratio. Such environmentally-induced females turn quickly to monoecism and, if selfed, they invariably breed true to monoecism.

All castor races respond to seasonal variations, irrespective of the stage of development of individual plants. In Israel, femaleness is strongest in spring and early sum-

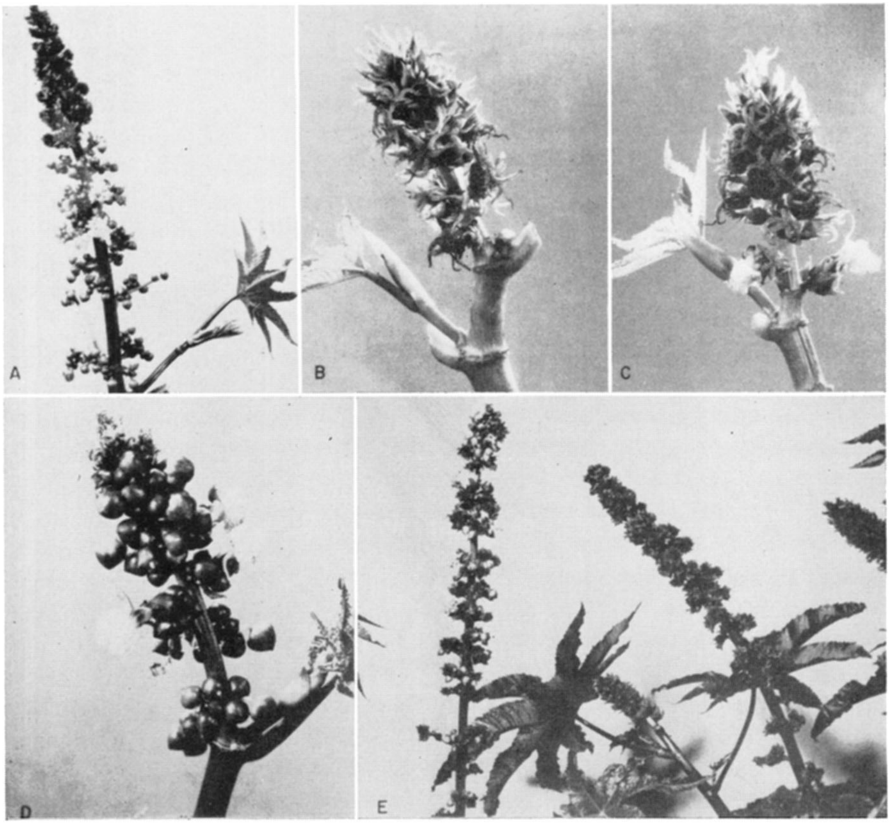

FIGURE 2.—Sex expression in different racemes of Ricinus. A, a normal raceme of the variety Herdocia; B, C, spring and winter racemes from the female inbred Nebraska; D, a raceme of the strongly male inbred 97 bearing a terminal hermaphrodite; E, female racemes from a sex-reversal plant of Queen 162; on the right a strictly pistillate raceme representing the prevailing type in spring and, on the left, a female raceme bearing interspersed staminate flowers representing the type occurring intermittently in winter.

mer, April to June. Strong male tendency is manifested intermittently during mid- and late-summer, July to September, and again during winter, January to February.

Genetic data on sex inheritance in castors were presented by KATAYAMA (1948) and CLAASSEN and HOFFMAN (1950). These workers describe a gynodioecious race in which monoecious and female plants occur in a ratio of 1:1, monoecious individuals being heterozygous. DR. C. E. CLAASSEN, formerly of the University of Nebraska, has generously furnished us with seed of such a backcross race, N145-4. Individual females of N145-4 produce intermittently a few staminate flowers at the base of some racemes during summer and later during winter (fig. 2, B and C). This behavior has enabled us to establish a female inbred known as Nebraska and to use its pollen in some crosses. Nebraska is strictly pistillate in spring and early summer in Israel.

As in a previous study of fruit color in Cucurbita (SHIFRISS 1949), a developmental approach was adopted in the present investigation of sex variation. Accordingly, the habit of growth and flowering of every plant tested was diagrammed with particular reference to time of appearance, sex expression, and position of each individual raceme. This developmental approach, in addition to breeding records, has helped to discern between hereditary and non-hereditary variations which often occur during the life of individual plants. It was quickly discovered that, unlike Nebraska which is quite stable phenotypically except for slight intermittent seasonal changes, the more common female variants revert sometime in their life to a permanent state of normal monoecism. Furthermore, in contrast to Nebraska, these sex-reversals do not breed true. With due consideration to the various facets of the problem, all sex forms have been divided into two major categories, according to their breeding behavior; genetically stable and genetically unstable sex forms.

The genetically stable forms include pistillate and monoecious variants all of which breed true. Under a given set of conditions, the homozygous monoecious races vary in their pistillate-staminate ratio from 2:1 to 1:14 (fig. 2, D). These races also differ from each other in their relative hereditary potentiality to form a terminal hermaphroditic flower at the apex of individual racemes, as well as in their ability to produce, under a given set of conditions, staminate and/or hermaphroditic flowers in the pistillate portion of the racemes. Our studies have confirmed and extended the findings of KATAYAMA and CLAASSEN and HOFFMAN. Accordingly, alleles of a major gene F differentiate between the various monoecious races, as well as between these races and the female inbred Nebraska, f; gene F is independent of the genes for plant color and waxy bloom but is linked with some of the polygenes which control the number of nodes preceding the primary raceme.

The genetically unstable forms consist of sex-reversals and monoecious individuals which do not breed true. These sex-reversals, which turn from femaleness to monoecism at different times in different regions of the same plant, can be divided further into various types and stocks depending on their relative capacity to generate sex-reversals. They also vary from each other in their ability to produce, under certain circumstances, interspersed staminate flowers in some of their pistillate and normal racemes. A fuller description of the phenotypic and breeding behavior of unstable stocks will be presented later.

INCIDENCE OF NATURALLY OCCURRING SEX-REVERSALS

An extensive search has been conducted for the occurrence of spontaneous pistillate plants in different castor populations which the writer has surveyed in Israel, Morocco, Kenya, Tanganyika, the Rhodesias, and in the Union of South Africa. Over a million plants of 48 different races were scanned. All female individuals found in this search proved to be sex-reversals, i.e., pistillate plants which are capable of reverting into *normal* monoecism sometime in their life (fig. 1,C–E).

Sex-reversals were discovered in practically all spontaneously grown populations, horticultural varieties, and artificially-developed inbred lines. The frequency of their occurrence is relatively high, but it varies considerably depending, among other things, on the breeding history of the material. In general, the frequency is higher in specially selected stocks and in open pollinated populations than in recently-bred monoecious strains.

TABLE 1

Incidence of naturally occurring sex-reversals

Variety	Total plants observed	Number sex-reversals	Frequency sex-reversals
Adamdam	40,000*	26	1:1,538
Baker No. 1	28,500	5	1:5,700
Blackwell	32,720	3	1:10,907
Cimarron	43,400	8	1:5,425
Early Spineless	82,000*	5	1:16,400
H.S. 1	1,500	4	1:375
Kehalhal	16,836	15	1:1,122
Oued Beth	250,000*	141	1:1,773

* Approximate number.

Some data on the frequency of occurrence of sex-reversals are given in table 1. Of special interest is the finding of 141 sex-reversals among a total of about 250,000 plants in the open pollinated Moroccan variety Oued-Beth, a frequency of about 1:1800. The incidence of sex-reversals in some of the newly introduced American varieties such as Baker No. 1, Blackwell and Cimarron is rather low. On the other hand, certain stocks of such varieties as U.S. 72, which consist of about 25 percent sex-reversals, have undoubtedly originated through selection of sex-reversals which often appear as superior yielding plants.

It is not unlikely that some of the naturally occurring sex-reversals are environmentally induced. Whether a specific sex-reversal is genetically controlled or not can be determined by its breeding behavior. Since breeding experiments, to be described later, have shown conclusively that the majority of the naturally occurring sex-reversals are genetically unstable, it can be concluded that the hereditary instability affecting sex, and manifested by the occurrence of sex-reversals, is a widely prevalent phenomenon in Ricinus. It is also significant that not a single genetically stable female mutant has been found in this extensive survey; therefore, a variant of the Nebraska type must be of extremely rare occurrence in nature.

PHENOTYPIC BEHAVIOR OF SEX-REVERSALS

All sex-reversals begin to bloom as females but sooner or later produce *normal* racemes. Such females may revert following the appearance of the primary pistillate raceme or after bearing over 500 pistillate inflorescences (fig. 1,C–F).

Phenotypic reversion does not occur in all the racemes of a plant simultaneously and some plants remain partly female and partly monoecious for several years. However, the earlier in the life of a female plant a given branch reverts, the sooner will the remaining shoots turn to monoecism. The first reverted raceme may be either normal or predominantly pistillate with a few staminate flowers at the base (fig. 1,B) and this phenotypic change to monoecism is developmentally irreversible. Therefore, femaleness in sex-reversals, or in any branch of a sex-reversal, can be viewed as a developmental phase, of varying duration, which always precedes monoecism as an end-product manifestation. Thus, unlike common fleeting variations, phenotypic reversion is a permanent change which appears to be transmitted lineally during development.

Under conditions favoring strong male tendency, interspersed staminate flowers may be found in the pistillate upper portion of normal racemes as well as in some pistillate inflorescences of sex-reversals. In spring, female racemes of all sex-reversals are strictly pistillate. In summer, interspersed staminate flowers appear intermittently on female racemes of some races of sex-reversals. However, in winter, there is a burst of interspersed staminate flowers on many female inflorescences of the same sex-reversals (fig. 2,E). Naturally, the presence of interspersed staminate flowers in pistillate racemes enables sex-reversals to reproduce themselves even before they revert to normal monoecism. Indeed, certain unstable stocks have already been reproduced in this manner for the past few years.

There exist wide racial differences in the ability to produce interspersed staminate flowers. Sex-reversals of the varieties Adom Mistaef and Queen 162 are quite prone to the production of interspersed staminate flowers. Some of the mutants described by CLAASSEN and HOFFMAN (1950) as Mendelian systems, as well as the interesting mutant described by BLARINGHEM, CHEVET and ROHLFS (1951) are probably similar sex-reversals. On the other hand, the sex-reversals of the variety Gamadon rarely bear interspersed staminate flowers. Whereas sex-reversals are related here to genetic instability, the occurrence of interspersed staminate flowers is a hereditary response to seasonal variations. Previous workers have not been aware of this important distinction between the two phenomena.

BREEDING BEHAVIOR OF SEX-REVERSALS

As pointed out earlier, sex-reversals and some of their monoecious descendants do not breed true. It should be emphasized that, as far as we were able to determine, this hereditary instability is not associated with any impairment of gametic fertility.

The naturally occurring sex-reversals represent a very heterogenous group of plants and they and their descendants can be divided conveniently into two general types, according to their phenotypic and breeding behavior. Type 1 embraces the great majority of the naturally occurring sex-reversals; these usually revert early in life, give, upon selfing, a relatively low percentage of sex-reversals in the offspring, less than 30 percent, and most of their progenies regress rapidly, in the course of a few generations, into stable monoecism. In contrast, type 2 represents rarely occurring sex-reversals that usually revert late in life and give, upon selfing, a relatively high percentage of sex-reversals, about 50 percent, which can be maintained easily and even increased appreciably by selection.

The above division of sex-reversals and unstable stocks into two general types was helpful in the early stages of our investigation and is still of value for characterizing the material used in various experiments. However, with increasing knowledge, we have come to regard all sex-reversals as a single graded series of genetically unstable individuals.

Reproduction of type 1

The breeding behavior of the great majority of naturally occurring sex-reversals is illustrated by the following example. The variety Adamdam, formerly N224A-2-1-3-2, has bred true in our trials to all its visible characteristics including monoecism.

When this inbred was grown on a large scale it proved again to be extremely uniform but consisted of 26 early reverted females in a population of about 40,000 plants. Of the 26 sex-reversals 24 were selfed and their offspring consisted, on the average, of 9 percent sex-reversals, ranging from 0 to 39 percent, based on a total of 899 plants. However, of the 24 progenies 9 consisted of monoecious offspring only. Selfing the remaining 15 lines for two additional generations resulted in a very low incidence in a few families, and in complete disappearance of sex-reversals in the majority. Although it is difficult to develop from such breeding material high sex-reversal producing stocks, it will be shown later that selection, when properly applied, can be effective even in type 1.

Naturally occurring sex-reversals were discovered in Adom Mistaef, Baker, Blackwell, Cimarron, Mautner, N201-2, R-248, U.S. 72 and in other varieties. Most of these females were early-reverted, i.e., they turned to monoecism before bearing their tenth raceme. However, a few exceptional individuals were also observed. These were late reverted females which produced very low percentages of sex-reversals.

Reproduction of type 2

Our first genetically unstable stocks of type 2 originated in two naturally occurring late reverted females found in distinctly different varieties. One mutant was discovered in 1950, in a dwarf variety of Far Eastern origin, and has been named Gamadon. The other mutant was discovered in 1952 in a large plantation of the open pollinated Moroccan variety, Oued Beth; this mutant is known as Queen 162. In contrast to sex-reversals of type 1, each of these two mutants produced over 50 percent of sex-reversals in the immediate offspring and the descendants have in general maintained the ability to produce high percentages of sex-reversals.

The Gamadon mutant was reproduced by selfing for six generations and thus far over 50,000 plants have been grown from it. This mutant breeds true to most visible characteristics, including small morphological structures, habit of growth, five nodes to the primary raceme, short internodes, and plant color. None of these characteristics has been affected during the course of inbreeding. In sex-reversals of Gamadon we have usually selfed normal racemes. However, it is also possible to self in a different manner, i.e., by the transfer of pollen from normal to pistillate inflorescences. In limited tests, no significant differences were noted between these two methods of selfing with regard to the percentage of sex-reversals in the offspring. Sibbing plays an important role in the reproduction of unstable stocks under natural conditions. By sibbing, we mean, in this case, the transfer of pollen from either sex-reversals or monoecious individuals to pistillate racemes of other sex-reversals of the same family. The results of artificial sibbing of this kind do not vary significantly from those obtained from selfing.

The data obtained from selfing of Gamadon for six consecutive generations can be summarized as follows: 1. Sex-reversals do not breed true and they usually differ from each other, in any selfed generation and in any family, in their individual ability to produce sex-reversals in the offspring. Although some sex-reversals are capable of producing 100 percent sex-reversals in their *immediate* offspring, others produce less than 30 percent sex-reversals. 2. Selection of certain individual sex-reversals in each generation can assure the maintenance of high sex-reversal producing stocks, however, once selection is halted, the percentage of sex-reversals drops steadily in each of the subsequent generations. 3. In any given unstable stock, monoecious individuals produce, on the average, a significantly lower percentage of sex-reversals in the offspring than do sex-reversal plants, but monoecious parents also differ greatly from each other in their ability to produce sex-reversals in the offspring; while some give monoecious offspring only, a few may produce over 50 percent sex-reversals. 4. Stable monoecious lines have been developed from high sex-reversal producing stocks.

Results similar to the above were obtained from selfing Queen 162 and other sex-reversals of type 2. Selfed seed was obtained in Queen 162 by three methods, i.e., selfing pistillate racemes bearing interspersed staminate flowers, selfing normal racemes, and selfing by transferring pollen from normal to pistillate racemes of the same sex-reversal plant. No consistently significant differences were observed among the three methods of selfing, with respect to the percentage of sex-reversals in the offspring.

Time of reversion—basis of selection

During the early stages of this study, in 1951, data were secured which indicated that, in unstable stocks of type 2, a relationship exists between *time* at which reversion occurs in sex reversals and their *ability* to produce sex-reversals in the offspring. However, it soon became apparent that, with the exception of extreme phenotypic categories, the breeding behavior of many sex-reversals of intermediate phenotypes cannot be predicted with a high degree of precision, on the basis of time of reversion. For example, in a given stock of Gamadon, sex-reversals were divided into three phenotypic groups: A. *Early*, females which reverted before the appearance of the tenth raceme; B. *Intermediate*, females which reverted between the tenth and the twentieth raceme; and C. *Late*, females which reverted after bearing over twenty pistillate inflorescences. Groups A (6 plants), B (9 plants), and C (12 plants) produced in their respective selfed progenies 46.4 percent sex-reversals based on 97 individuals, 43.7 percent based on a total of 231 individuals, and 60.4 percent based on 310 plants. Within each group, phenotypically similar individuals may produce significantly different percentages of sex-reversals, but in group C, exceedingly late reverted females, which turn to monoecism after bearing over 100 pistillate racemes, always produce close to 100 percent sex-reversals in their offspring. It is now realized that the above classification into three groups is inadequate since it arbitrarily limits the range of phenotypes in group B and includes in group C an extremely wide range of variants, from females which turn to monoecism between the 20th and the 25th racemes to others which revert after bearing over 500 pistillate racemes! Nevertheless, on the basis of this classification a more consistent correlation was found between time of reversion and breeding behavior when the latter was measured by the percentage of sex-reversals obtained in the F_1 offspring. Crosses were made between pistillate racemes of phenotypically different sex-reversals of groups A, B, and C of Gamadon with the genetically stable monoecious inbred, Early Spineless. The F_1 of group A (22 parents) consisted of 12.4 percent sex-reversals based on a total of 2278 plants, the F_1 of group B (29 parents) consisted of 28.9 percent based on 4058 plants, and the F_1 offspring of group C (17 parents) consisted of 52.2 percent sex-reversals based on a total of 2104 individuals. A closer examination of data revealed the existence of some differences in breeding behavior between phenotypically similar individual parents, but all exceedingly late reverted females produced close to 100 percent sex-reversals in crossing as they do in selfing.

In terms of years of growth, exceedingly late reverted females are quite variable in regard to time at which they turn to monoecism. It is believed that the hereditary potentialities of exceedingly late reverted females should be measured not by the percentage of sex-reversals obtained in the immediate offspring—which is usually close to 100 percent—but primarily by the rate of decline in the percentage of sex-reversals for a number of generations, following selfing. This has not been done yet. Fortunately, sex-reversals which are female for an extended period—some sex-reversals may not revert during their life span (about 10 years)—can be selfed by interspersed staminate flowers and the "rate of decline" of each may be obtained experimentally.

It has been demonstrated that any monoecious inbred can be converted into a high sex-reversal producing race either directly from a rare occurring late reverted female of type 2, or indirectly from the more commonly occurring sex-reversals of type 1. The conversion of type 1 into type 2 can be accomplished in two or three generations provided, following selfing, a sufficiently large number of sex-reversals is screened. For example, a naturally occurring sex-reversal of the local variety Adom Mistaef produced upon selfing about 5 percent sex-reversals, based on a population of 860 plants. Most of these sex-reversals were early reverted and they produced very low percentages of sex-reversals in the second inbred generation, but there was one tree which reverted after bearing 388 pistillate racemes and the 145 offspring of that tree consisted of 144 sex-reversals and one monoecious individual. In a similar manner, a high sex-reversal producing stock was developed from individuals of the second inbred generation of the sex-reversals of Adamdam. Once a given individual produces, upon selfing, over 50 percent sex-reversals, it is certain that this offspring is of type 2.

Data are presented in table 2 on the effect of selection for early and late occurring reversions in families of four different varieties. These data show that late reverted females give high percentages of sex reversals which are mostly late reverted; and early reverted females give low percentages of sex reversals which are mostly early reverted. The data in table 2 also show that early reverted females of Gamadon produce, on the average, a higher percentage of sex-reversals than phenotypically similar females of other varieties. This strengthens our general contention that Gamadon has a remarkable ability to generate sex-reversals.

It is concluded from the above that the time of reversion is under hereditary control and that in unstable stocks of type 2 the later in life a plant reverts the higher is its potentiality to produce sex-reversals in the offspring.

Inheritance

In crosses between pistillate racemes of sex-reversals and genetically stable races, female and monoecious, the resulting individual F_1's consist of from 0 to 100 percent sex-reversals depending primarily on the particular sex-reversal parent employed, although the influence of the stable variety cannot be ignored, as will be shown later. In a given unstable stock of type 2, crossing *monoecious* plants with stable races gives,

TABLE 2

Selection for and transmission of time of sex reversion in unstable varieties

Parental material		Offspring						
Unstable variety	Phenotypes selfed	Number monoecious plants	Number early reverted females	Number late reverted females	Total sex-reversals	Total number plants	Percent late reverted females	Percent sex-reversals
Adamdam	7 exceedingly late reverted females	12	15	1546	1561	1573	98.3	99.2
	10 early reverted females	860	157	51	208	1068	4.8	19.5
Adom Mistaef	1 exceedingly late reverted female	1	3	140	144	145	96.6	99.3
	7 early reverted females	404	49	6	55	459	1.3	12.0
Gamadon	18 late reverted females	787	—	—	4841	5628	—	86.0
	9 early reverted females	272	—	—	191	463	—	41.3
Queen 162	22 late reverted females	60	13	1184	1197	1257	94.2	95.2
	10 early reverted females	553	33	2	35	588	0.34	6.0

on the average, a significantly lower percentage of sex-reversals in the F_1 than crossing *sex-reversals* with the same stable varieties. It should be mentioned, however, that at least in one case, the F_1 of a cross between a monoecious plant of type 2, Gamadon, and the stable monoecious inbred, Early Spineless, consisted of 19 monoecious and 11 sex-reversal plants. This particular monoecious plant of Gamadon gave upon selfing over 50 percent sex-reversals in the offspring.

In general, heterozygous sex-reversals revert earlier in life than their sex-reversal parents and earlier than the sex-reversal offspring obtained from selfing of these parents. But heterozygous sex-reversals vary greatly in time of phenotypic reversion, as do sex-reversals in self-reproducing stocks, and in crosses between exceedingly late reverted females and stable races, one is likely to find a few exceedingly late reverted females in the F_1. In such crosses, the F_1 invariably consists of over 90 percent sex-reversals. It therefore appears that the phenotype of sex-reversal is dominant over the phenotype of stable races. Is this "dominance" due to gene action or to cytoplasmic transmission?

The discovery of mutant Queen 162, which bears interspersed staminate flowers during some periods of the year, has enabled us to test the nuclear and cytoplasmic hypotheses. Pollinating stable races, female and monoecious, with pollen from interspersed staminate flowers borne on pistillate racemes of exceedingly late reverted females of Queen 162 results in over 90 percent sex-reversals in the F_1. This proves that the factor for sex-reversal can be transmitted fully through male gametes. ZIMMERMAN and PARKEY (1954) reported that crossing females of the backcross line N145-4 with certain varieties gave 100 percent females in the F_1. It is not unlikely that one of the pollen parents employed by these workers was a sex-reversal and so was the F_1.

Paired reciprocal crosses also proved that sex instability is under nuclear control. These reciprocal crosses were made within pairs of parental racemes. Each pair consisted of one pistillate raceme, bearing interspersed staminate flowers, of a sex-reversal of Queen 162, and one normal raceme of the stable monoecious variety, Kehalhal. Pollen was exchanged within each pair. The F_1 can be readily distinguished from selfs on the basis of plant color; Queen 162 being red non-waxy, Kehalhal green waxy, and the F_1 red waxy. The data from nine such pairs indicated that pollen from interspersed staminate flowers of a given pistillate raceme transmits to the F_1 offspring the factor for sex instability not less effectively than ovules of the same raceme.

The percentage of sex-reversals in backcrosses, F_1 (sex-reversals × stable races) × stable races, and in F_2's is usually very low and it drops rapidly in subsequent generations. Individual F_2 progenies vary from each other and, in general, monoecious F_1 gives a slightly lower percentage of sex-reversals than sex-reversal F_1. The low sex-reversal potential of the majority of the naturally occurring female individuals may be due, among other factors, to heterozygosity.

Pistillate racemes of exceedingly late reverted females of Queen 162 were crossed with the monoecious variety Kehalhal. The F_1 consisted of 93 percent sex-reversals, based on a total of 2,858 individuals, and the F_2 consisted of 26 percent sex-reversals, based on a total of 1,720 plants. Late reverted females of Gamadon were crossed with the monoecious inbred, Early Spineless. The F_1 consisted of 70 percent sex-reversals and the F_2 of about 12 percent (table 3). If the parental sex-reversal is of type 1, the percentage of sex-reversals in the F_2 may be very low, often less than one percent.

TABLE 3

Inheritance of sex-reversal

Cross	F_1			F_2			
	Phenotype	Number plants observed	Number plants selfed	Number monoecious plants	Number sex-reversals	Total number plants	Percent sex-reversals
Sex-reversals, variety Gamadon × Monoecious variety E. Spineless	Monoecious	56	5	107	0	107	0.0
				106	3	109	2.8
				104	3	107	2.8
				92	11	103	10.7
				94	4	98	4.1
	Total for monoecious F_1			503	21	524	4.0
	Sex-reversals	142	7	84	16	100	16.0
				104	5	109	4.6
				92	17	109	15.6
				69	24	93	25.8
				29	12	41	29.3
				60	1	61	1.6
				57	45	102	44.1
	Total for sex-reversals F_1			495	120	615	19.5
	Grand total			998	141	1139	12.4

Crosses were also made between pistillate racemes of late reverted females of Gamadon and the female inbred Nebraska. Of 51 F_1 plants, 38 were sex-reversals and 13 monoecious individuals. All the 51 F_1 plants were selfed. The F_2 obtained from the sex-reversal F_1 consisted of 30 percent females based on 231 offspring and the F_2 obtained from the monoecious F_1 consisted of 28 percent females based on a total of 3,092 plants. A check on the breeding behavior of the F_2 female segregants showed that there were 25 percent females of the Nebraska type and about 4 percent sex-reversals among the entire F_2 population.

The above data suggest that crossing between stable and unstable races hastens regression towards phenotypic and genetic stability, from F_2 on.

FACTORS AFFECTING INCIDENCE OF SEX-REVERSALS

Pistillate racemes of late reverted females of Gamadon were pollinated by a mixture of pollen obtained from our inbred 97, pistillate-staminate ratio 1:14, and from Early Spineless, pistillate-staminate ratio 1:2. The two F_1 offspring can be distinguished by plant color and waxy bloom. The F_1 Gamadon $\times$ 97 consisted of 91 monoecious and 168 sex-reversal plants and the F_1 Gamadon $\times$ Early Spineless consisted of 23 monoecious plants and 192 sex-reversals. The difference between the two F_1 offspring is highly significant, P being less than 0.01.

In the spring of 1952, the variety Oued-Beth was planted on a large scale by the French firm Organico in two ecologically distinct regions of Morocco; in Nouasseur near Casablanca, relatively mild climate and fairly good soils, and in Aghouatim, close to Marrakech, desert climate and submarginal soils. At the time of full bloom, the average pistillate-staminate ratio in the Nouasseur plantation was 1:2 and sex-reversals were found there at a rate of 1:1500, based on about 100,000 plants. In the Aghouatim plantation, the average pistillate-staminate ratio was 1:7 and not a single sex-reversal plant was found among about the same number of plants as in Nouasseur.

Seed of an unstable stock of Queen 162 was sown on November 11, 1954, and again on March 15, 1955, near Hadera, Israel. The winter planting was in full bloom on March 5, 1955, and of 643 plants, 287 were monoecious and 356 were sex-reversals. The spring planting was in full bloom on June 12, 1955 and of 615 plants, 72 were monoecious and 543 were sex-reversals. The difference between the two fields in regard to the incidence of sex-reversals is highly significant, P being less than 0.01. In spite of these results, certain stocks which consist of close to 100 percent sex-reversals are not affected by date of sowing.

It is interesting that factors which favor strong male tendency may also decrease the incidence of sex-reversals in some stocks.

ON THE NATURE OF SEX REVERSION

A number of breeding experiments were conducted in order to obtain some data bearing upon the nature of the phenotypic change in sex-reversals. In one of the tests, both pistillate and emasculated monoecious racemes of individual sex-reversals of Gamadon were pollinated by the monoecious variety Early Spineless. The F_1 obtained from the pistillate racemes consisted, on the average, of 30.3 percent of sex-reversals, based on a total of 2,081 plants, and the F_1 progeny obtained from the emasculated racemes consisted of only 2.7 percent sex-reversals, based on a total of 1,213 plants.

If the phenotypic change from femaleness to monoecism is associated with a corresponding hereditary change, as the data suggest, how can one explain the fact that selfing monoecious racemes of exceedingly late reverted females of type 2 often gives about 100 percent sex-reversals in the immediate offspring?

Pistillate racemes of five late reverted females of Gamadon were crossed, during winter, with the stable female inbred, Nebraska. Of 164 F_1 plants, 66 percent were sex-reversals. The reciprocal cross was produced, in spring, in an isolation field by planting Nebraska as seed parent, in alternate rows with Gamadon. The monoecious plants and the early reverted females were pulled out from the rows of Gamadon before Nebraska started flowering; thus, the stable female inbred was pollinated by wind-borne pollen carried from the monoecious racemes of late reverted females of Gamadon. The two parents involved in this cross are green non-waxy and the F_1 progeny can always be recognized from selfs by its waxy appearance (gene interaction). Of 781 plants grown from this reciprocal cross, only 6.5 percent were sex-reversals. However, open pollinated (intrabreeding) seed saved from the monoecious racemes of the late reverted females, used as male parents in this cross, produced over 75 percent sex-reversals based on a total 980 plants. If 6.5 percent of sex-reversals in the F_1 corresponds approximately to the percentage of gametes carrying the reversion factor in the monoecious racemes of the sex-reversal parent, we should have expected that selfing or open pollination of such racemes would give about 13 percent sex-reversals instead of 75 percent actually observed.

More comparable data were obtained from a carefully designed paired three-way pollination test. In this test, monoecious racemes of late-reverted females of Gamadon and pistillate racemes bearing a few staminate flowers at the base of Nebraska were employed. Each operation involved a single pair of parental racemes within which three different pollinations were made; reciprocal crosses as well as selfing of the monoecious raceme of the sex-reversal parent. The pooled data obtained from 25 such pairs show that reciprocal crosses between monoecious racemes of sex-reversals and pistillate racemes of the genetically stable females do not differ significantly. However, while the F_1 offspring consisted of 14 percent sex-reversals, based on a total of 697 plants, the offspring obtained from selfing consisted of 75 percent sex-reversals, based on a total of 143 plants. Again, if 14 percent sex-reversals in the F_1 corresponds to the percentage of gametes carrying the reversion factor in the monoecious racemes of the sex-reversal parent one should have expected that selfing such racemes would give about 26 percent sex-reversals instead of 75 percent actually observed.

Another paired three-way pollination test was made, this time involving late reverted females of Gamadon and the monoecious variety Conner; crossing gave less than 2 percent of sex-reversals, while selfing resulted in about 70 percent.

Thus, the data from crossing of sex-reversals suggest that the phenotypic change from femaleness to monoecism is accompanied by a corresponding genetic change while data from selfing do not allude such a change. One may argue that penetrance of sex-reversals in the F_1 is lower than in the offspring obtained from selfing. However, this argument is untenable because, employing the same stable races, crossing early appearing *pistillate* racemes of exceedingly late reverted females of type 2 often results in close to 100 percent sex-reversals in the F_1.

Any interpretation of the nature of the phenotypic change in sex-reversals must account for the apparent paradox which the above results have brought to light.

HYPOTHESIS

It is evident from the phenotypic and breeding behavior of sex-reversals and their descendants that sex instability in castors represents a case of the "eversporting" or "variegation" phenomenon. The data show that in sex-reversals of unstable stocks the time of change from femaleness to monoecism is largely under nuclear control and the remarkable efficacy of selection for late occurring reversions recalls the action of "mutable loci" as interpreted by McClintock (1951). In the absence of critical cytogenetic evidence, it is perhaps superfluous to offer a sound interpretation of sex instability in castors. Nevertheless, the observations on the behavior of sex-reversals and the results from various breeding tests, projected in the light of available knowledge, have prompted the presentation of a tentative genetic hypothesis.

In monoecious races, gene F (or the genotypic complex controlling monoecism) exerts its full impact extremely early in ontogeny. In sex-reversals, an unstable nuclear factor brings about the suppression of F thereby delaying gene impact, inhibiting male potentiality, and resulting in individuals which begin to bloom as females. The nuclear factor mutates into different suppressors varying in degrees of suppression and stability. These mutations are reversible. The suppression of F creates a delicate balance between a number of factors, and the *time* at which gene impact occurs depends on the dose of the suppressor, the potential sex tendency of F, and non-genetic variations. The higher the degree of suppression of F, the less noticeable is the effect of environmental conditions upon the incidence of sex-reversals.

Phenotypic reversion occurs when the suppressed F is released and the majority of the cells in the sex differentiating tissue are homozygous for released F. The release of F may or may not be associated with the loss of the suppressor. A released F can be suppressed again and this suppression can be manifested in femaleness if, following fertilization, an effective suppressor is present in a homozygous condition. According to this hypothesis, the expression of F is cyclically controlled by the suppressor and, theoretically at least, sex-reversals could breed true if an effective suppressor became stable. The paradox arising from the paired three-way pollination test is resolved by assuming that, in the F_1, sex-reversals $\times$ stable races, zygotes carrying a suppressed F from the sex-reversal parent are likely to develop into sex-reversals but comparable zygotes carrying a released F are usually monoecious.

The results of crosses between sex-reversals and stable monoecious races may lead one to conclude that femaleness is dominant over monoecism. However, according to our hypothesis, what is dominant or partially dominant in the F_1 is the delay in gene impact.

While the developmental course of sex-reversals is due to suppression and subsequent release of gene F by an unstable nuclear factor, other phenotypic, usually intermittent, changes which occur during development are attributed to non-genetic fluctuations.

SUMMARY

In the castor-oil plant, gene *F*, for monoecism, controls a genetically *stable* series of sex variants ranging from female, *f*, to strongly male inbreds. The sex expression of these strains is subject to non-genetic variations which occur *intermittently* during the development of individual plants.

Sex-reversals occur spontaneously in many natural populations and inbred races. They represent a genetically *unstable* series of females differing in *time* of life at which they turn to monoecism, as well as in *ability* to produce sex-reversals in the offspring. This phenotypic change from femaleness to monoecism is developmentally *irreversible*.

The offspring of sex-reversals consist of varying proportions of sex-reversals and monoecious individuals. In general, early reverted females produce relatively low percentages of females which are mostly early reverted and exceedingly late reverted females produce over 90 percent females which are mostly late reverted. By rigid selection for early or late occurring reversions, any self-reproducing unstable stock can be converted into either low or high sex reversal producing lines. Once selection is halted, a rapid regression occurs towards stable monoecism in the subsequent generations. This indicates that the mutation in question is reversible but that the main direction is towards monoecism.

The data show that the *time* of sex reversion is under nuclear control. However, nothing is known at present about the nature and location of the unstable nuclear material involved.

Although the incidence of sex-reversals in any stock depends largely upon an unstable nuclear material, it is also influenced by genetic and non-genetic factors affecting sex tendency.

A hypothesis is presented to account for sex instability in castors.

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